CARDIOVASCULAR MEDICINE

# The influence of age, sex and other variables on the plasma level of N-terminal pro brain natriuretic peptide in a large sample of the general population

**I Raymond, B A Groenning, P R Hildebrandt, J C Nilsson, M Baumann, J Trawinski, F Pedersen**

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See end of article for authors' affiliations

Correspondence to: Dr Bjoern A Groenning, Department of Cardiology and Endocrinology, Copenhagen University Hospital Frederiksberg, 57 Nordre Fasanvej, DK-2000 Frederiksberg, Denmark; bjoerng@dadlnet.dk

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**Objective:** To identify potentially confounding variables for the interpretation of plasma N-terminal pro brain natriuretic peptide (NT-proBNP).
**Design:** Randomly selected subjects filled in a heart failure questionnaire and underwent pulse and blood pressure measurements, ECG, echocardiography, and blood sampling.
**Setting:** Subjects were recruited from four Copenhagen general practices located in the same urban area and were examined in a Copenhagen University Hospital.
**Patients:** 382 women and 290 men in four age groups: 50–59 years (n = 174); 60–69 years (n = 204); 70–79 years (n = 174); and > 80 years (n = 120).
**Main outcome measures:** Associations between the plasma concentration of NT-proBNP and a range of clinical variables.
**Results:** In the undivided study sample, female sex ($p < 0.0001$), greater age ($p < 0.0001$), increasing dyspnoea ($p = 0.0001$), diabetes mellitus ($p = 0.01$), valvar heart disease ($p = 0.002$), low heart rate ($p < 0.0001$), left ventricular ejection fraction ⩽ 45% ($p < 0.0001$), abnormal ECG ($p < 0.0001$), high $\log_{10}$[plasma creatinine] ($p = 0.0009$), low $\log_{10}$[plasma glycosylated haemoglobin A1c] ($p = 0.0004$), and high $\log_{10}$[urine albumin] ($p < 0.0001$) were independently associated with a high plasma $\log_{10}$[plasma NT-proBNP] by multiple linear regression analysis.
**Conclusions:** A single reference interval for the normal value of NT-proBNP is unlikely to suffice. There are several confounders for the interpretation of a given NT-proBNP concentration and at the very least adjustment should be made for the independent effects of age and sex.

Natriuretic peptides are gaining increased recognition as diagnostic markers in heart failure, and brain natriuretic peptide (BNP)[1 2] and its amino terminal portion, N-terminal pro brain natriuretic peptide (NT-proBNP)[3]—which may have advantages because of its greater stability[4]—have proved especially promising. Concentrations of BNP and NT-proBNP are related to left ventricular filling pressures[5] and wall stress.[6]

All previous studies of the diagnostic value of BNP in heart failure, whether focusing purely on left ventricular systolic dysfunction[7–10] or on a clinical diagnosis of heart failure,[11 12] have consistently reported a very high negative predictive value of BNP (0.98 to 1.00), while the positive predictive value has been lower (0.16 to 0.42). The high negative predictive value makes BNP appear well suited for population screening; however, the low positive predictive value may pose a problem in any population screening or diagnostic setting because of the large number of false positive results that are likely to be found.

Our aim in this population based study was to try to identify potentially confounding variables for the interpretation of the plasma concentration of NT-proBNP, which might lead to better diagnostic performance of the marker.

## METHODS

### Patients

The study sample was recruited from four Copenhagen general practitioners located in the same urban area. The sole inclusion criterion was age between 50–90 years. Exclusion criteria were inability to cooperate (for example, because of dementia), residence in a nursing home, and lack of response to two written invitations.

### Study design

In order to obtain a sufficient number of elderly subjects, the study sample was stratified to include at least 120 subjects in each decade. An invitation to participate in the study was sent to all persons between 50–90 years of age assigned to the first two general practitioners. From the third practice, all persons aged 60–90 years were invited, and from the last practice, all persons aged 80–90 years. Seventy per cent of those invited participated in the study. The attendance was higher among younger individuals (approximately 75%), while it was approximately 50% in the oldest decade. The study was designed to be representative of the background population in Copenhagen by compensating for the lower response rate in the older age groups through oversampling in those groups.

Subjects from general practitioners 1 and 2 entered the study in the autumn of 1997; they completed a questionnaire and had echocardiography and a blood pressure measurement. They were recalled by the autumn of 1998 (n = 424), when they had an ECG and a blood sample was taken. Subjects from general practitioners 3 (n = 207) and 4 (n = 41) had all the investigations in the autumn and winter of 1998-99. Thus all the subjects in the study had each investigation done once.

The local ethics committee approved the study and the patients gave their written informed consent.

### Heart failure questionnaire

All subjects filled in a heart failure questionnaire on their medical history, symptoms, drug history, and alcohol and tobacco consumption. Symptoms of heart failure were recorded from questions on ankle swelling and breathlessness (self reported). The degree of breathlessness was recorded

**Table 1** Clinical characteristics of the subjects in the study

| Characteristic | All subjects (n=672) | "Normal" subjects¶ (n=130) |
|---|---|---|
| *Demography* | | |
| Men/women | 290 (43%) / 382 (57%) | 53 (41%) / 77 (59%) |
| Age (years) | | |
| 50–59 | 174 (26%) | 59 (45%) |
| 60–69 | 204 (30%) | 45 (35%) |
| 70–79 | 174 (26%) | 19 (15%) |
| ⩾80 | 120 (18%) | 7 (5%) |
| Median (5th to 95th centiles) | 68.1 (52.6 to 86.1) | 61.0 (51.8 to 80.4)‡ |
| Current daily smoker | 237 (35%) | 51 (39%) |
| *Clinical history* | | |
| Dyspnoea¶¶ | | |
| Grade 2 | 84 (13%) | 11 (8%) |
| Grade 3 | 51 (8%) | 6 (5%) |
| Grade 4 | 47 (7%) | 1 (1%) |
| Grade 5 | 14 (2%) | 0 (0%) |
| Grade 6 | 23 (3%) | 1 (1%) |
| Median (5th to 95th centiles) | 1 (1 to 5) | 1 (1 to 3)‡ |
| Ankle oedema | 155 (23%) | 17 (13%)† |
| Ischaemic heart disease | 96 (14%) | – |
| Hypertension | 169 (25%) | – |
| Angina | 83 (12%) | – |
| Diabetes mellitus | | |
| Insulin treated | 7 (1%) | – |
| Non-insulin dependent | 36 (5%) | – |
| Pulmonary disease | 74 (11%) | – |
| Valvar heart disease | 10 (1%) | – |
| *Measurements*§ | | |
| Heart rate (beats/min) | 75.4 (74.4 to 76.4) | 73.8 (71.7 to 75.8) |
| Systolic blood pressure (mm Hg) | 144 (142 to 145) | 124 (122 to 126)‡ |
| Diastolic blood pressure (mm Hg) | 86.6 (85.6 to 87.5) | 79.2 (77.9 to 80.4)‡ |
| Pulse pressure (mm Hg) | 56.9 (55.4 to 58.3) | 44.8 (43.0 to 46.5)‡ |
| Left ventricular ejection fraction (%) | | |
| ⩽50 | 77 (11%) | – |
| ⩽45 | 58 (9%) | – |
| ⩽40 | 38 (6%) | – |
| ⩽35 | 21 (3%) | – |
| Median (5th to 95th centiles) | 60 (40 to 60) | 60 (60 to 60)‡ |
| NT-proBNP (pmol/l) (geometric mean) | 35.7 (32.8 to 38.9) | 18.7 (15.9 to 22.1)‡ |
| HbA1c (%) (geometric mean) | 5.60 (5.55 to 5.66) | 5.38 (5.31 to 5.46)‡ |
| Creatinine (μmol/l) (geometric mean) | 84.6 (83.3 to 85.8) | 82.1 (79.8 to 84.4)* |
| Urine albumin (%) (geometric mean) | 9.06 (8.35 to 9.82) | 6.13 (5.33 to 7.04)‡ |
| Atrial fibrillation (n (%)) | 24 (4) | – |
| Abnormal ECG¶¶¶ (n (%)) | 197 (29) | – |
| *Drug treatment*§§ | | |
| Diuretics | | |
| Loop | 37 (6) | – |
| Others | 82 (12) | – |
| ACE inhibitor | 42 (6) | – |
| Angiotensin II blocker | 12 (2) | – |
| β Blocker | 30 (5) | – |
| Calcium antagonist | 39 (6) | – |
| Nitrates | 26 (4) | – |
| Digoxin | 25 (4) | – |
| Aspirin | 112 (17) | 10 (8)† |

Differences between groups are not significant unless indicated. *p<0.05; †p<0.005; ‡p<0.0001, "normal" *v* remainder of subjects.
¶Definition of "normal": no congestive heart failure, no ischaemic heart disease, no history of hypertension, no diabetes, no lung disease, no cardiovascular drug treatment, left ventricular ejection fraction ⩾ 60%, blood pressure <140/90 mm Hg, and a normal ECG.
¶¶Dyspnoea classified according to WHO: grade 2, dyspnoea on vacuum cleaning or climbing one flight of stairs; grade 3, dyspnoea when walking on an even road; grade 4, dyspnoea on minimal exertion; grade 5, orthopnoea; grade 6, dyspnoea at rest.
¶¶¶Abnormal ECG: presence of arrhythmias, ST deviation, Q waves, hypertrophy, or abnormal left ventricular axis (according to the Minnesota code).
§Values are mean (95% confidence interval) unless indicated.
§§Values are n (%).
ACE, angiotensin converting enzyme; HbA1c, glycosylated haemoglobin; NT-proBNP, N-terminal pro brain natriuretic peptide.

from the questionnaire using the World Health Organization classification (grade 1, no dyspnoea; grade 2, dyspnoea when vacuum cleaning or climbing stairs to the next floor; grade 3, dyspnoea when walking on an even road; grade 4, dyspnoea on minimum exertion; grade 5, orthopnoea; grade 6, dyspnoea at rest). Self reported medical history was recorded from questions on hospital admissions (with special emphasis on admissions for heart failure, pulmonary oedema, and

**Table 2** Variables independently associated with the plasma concentration of N-terminal pro brain natriuretic peptide

| Variable included in multiple linear regression models | Regression coefficient: all subjects (n=672) | Regression coefficient: "normal" subjects¶ (n=130) |
|---|---|---|
| Sex (0=female; 1=male) | –0.177 (0.031), p<0.0001 | –0.197 (0.061), p=0.002 |
| Age (years) | 0.018 (0.002), p<0.0001 | 0.020 (0.003), p<0.0001 |
| Dyspnoea¶¶ | 0.045 (0.012), p=0.0001 | NS |
| Ankle oedema | NS | NS |
| Ischaemic heart disease | NS | – |
| Hypertension | NS | – |
| Diabetes mellitus | 0.175 (0.068), p=0.01 | – |
| Pulmonary disease | NS | – |
| Valvar heart disease | 0.370 (0.119), p=0.002 | – |
| Heart rate (beats/min) | –0.005 (0.001), p<0.0001 | NS |
| Systolic blood pressure (mm Hg) | NS | NS |
| Diastolic blood pressure (mm Hg) | NS | –0.012 (0.004), p=0.007 |
| Pulse pressure (mm Hg) | NS | NS |
| LV ejection fraction ⩽45% | 0.207 (0.052), p<0.0001 | – |
| Abnormal ECG¶¶¶ | 0.180 (0.033), p<0.0001 | – |
| $\log_{10}$[plasma creatinine] (μmol/l) | 0.262 (0.079), p=0.0009 | NS |
| $\log_{10}$[plasma HbA1c] (%) | –0.473 (0.132), p=0.0004 | NS |
| $\log_{10}$[urine albumin] (%) | 0.085 (0.015), p<0.0001 | NS |

Values are regression coefficient estimates (SE). Dependent variable: $\log_{10}$[N-terminal pro brain natriuretic peptide] (pmol/l). Intercepts (SE) in model including all subjects and in model including only normal subjects were 0.020 (0.397) (NS) and 1.066 (0.441) (p = 0.02), respectively. Coding of dichotomous variables: 0, absence of condition; 1, presence of condition.
¶Definition of "normal": no congestive heart failure, no ischaemic heart disease, no history of hypertension, no diabetes, no lung disease, no cardiovascular drug treatment, left ventricular ejection fraction ⩾60%, blood pressure < 140/90, and a normal ECG.
¶¶Dyspnoea classified according to WHO: grade 2, dyspnoea on vacuum cleaning or climbing one flight of stairs; grade 3, dyspnoea when walking on an even road; grade 4, dyspnoea on minimal exertion; grade 5, orthopnoea; grade 6, dyspnoea at rest.
¶¶¶Abnormal ECG: presence of arrhythmias, ST deviation, Q waves, hypertrophy, or abnormal left ventricular axis (according to the Minnesota code).
HbA1c, glycosylated haemoglobin; LV, left ventricular.

myocardial infarction), history of ischaemic heart disease (previous myocardial infarction and/or angina), history of hypertension, and history of diabetes mellitus (insulin treated or non-insulin-dependent). Each questionnaire was evaluated immediately after it was completed and where there were any omissions or inconsistencies the subjects were contacted by telephone to ensure the highest possible data quality.

### Measurements

Heart rate and blood pressure were measured and a 12 lead standard ECG was recorded and evaluated according to the Minnesota code. Using predefined criteria (absence of arrhythmias, ST deviation, Q waves, hypertrophy, or abnormal left ventricular axis), each ECG was classified as either normal or abnormal.

**Table 3** Geometric mean concentrations of N-terminal pro brain natriuretic peptide in different age and sex groups in the undivided study sample

| Age (years) | Sex | N | NT-proBNP (pmol/l)* | | Age (years)** | |
|---|---|---|---|---|---|---|
| All | All | 672 | 35.7 (32.8 to 38.9) | p<0.0001† | 68.1 (52.6 to 86.1) | p=0.0006‡ |
| | Women | 382 | 42.6 (38.2 to 47.4) | | 69.9 (52.8 to 87.2) | |
| | Men | 290 | 28.5 (25.0 to 32.6) | | 65.2 (52.5 to 83.1) | |
| 50–59 | All | 174 | 16.1 (14.0 to 18.6)§ | p=0.05† | 54.9 (51.5 to 58.8) | NS‡ |
| | Women | 90 | 18.3 (15.3 to 21.8) | | 55.2 (51.4 to 58.8) | |
| | Men | 84 | 14.2 (11.4 to 17.5) | | 54.6 (51.7 to 58.7) | |
| 60–69 | All | 204 | 27.2 (24.1 to 30.5)§ | p=0.002† | 64.8 (60.7 to 69.5) | NS‡ |
| | Women | 103 | 32.6 (27.8 to 38.3) | | 64.7 (60.7 to 69.5) | |
| | Men | 101 | 22.5 (19.1 to 26.6) | | 64.8 (60.7 to 69.5) | |
| 70–79 | All | 174 | 51.4 (44.8 to 59.0)§ | NS† | 75.0 (70.7 to 79.6) | p=0.03‡ |
| | Women | 102 | 54.9 (45.9 to 65.6) | | 74.6 (70.8 to 78.7) | |
| | Men | 72 | 46.9 (37.8 to 58.1) | | 75.9 (70.7 to 79.8) | |
| ⩾80 | All | 120 | 108 (90.5 to 128)§ | NS† | 84.1 (80.3 to 89.8) | NS‡ |
| | Women | 87 | 104 (85.6 to 126) | | 84.5 (80.3 to 90.1) | |
| | Men | 33 | 118 (81.2 to 172) | | 82.6 (80.7 to 88.5) | |

*Geometric mean (95% confidence interval).
**Median (5th to 95th centiles).
§Trend in the plasma concentration of NT-proBNP over age groups analysed by analysis of variance: p<0.0001.
†Two sample *t* test; ‡Mann–Whitney test, women *v* men.
NT-proBNP, N-terminal pro brain natriuretic peptide.

**Table 4** Geometric mean concentrations of N-terminal pro brain natriuretic peptide in different age and sex groups in subjects defined as normal in the study sample¶

| Age (years) | Sex | N | NT-proBNP (pmol/l)* | | Age (years)** | |
|---|---|---|---|---|---|---|
| All | All | 130 | 18.7 (15.9 to 22.1) | | 61.0 (51.8 to 80.4) | |
| | Women | 77 | 22.1 (18.0 to 27.1) | p<0.02† | 59.0 (51.9 to 80.4) | NS‡ |
| | Men | 53 | 14.8 (11.3 to 19.3) | | 61.6 (51.6 to 82.4) | |
| 50–59 | All | 59 | 13.1 (10.4 to 16.6)§ | | 54.4 (51.5 to 58.7) | |
| | Women | 39 | 15.0 (11.5 to 19.6) | NS† | 54.4 (51.5 to 58.8) | NS‡ |
| | Men | 20 | 10.1 (6.4 to 16.0) | | 54.7 (51.4 to 57.2) | |
| 60–69 | All | 45 | 18.4 (14.5 to 23.5)§ | | 63.5 (60.7 to 68.9) | |
| | Women | 23 | 25.8 (19.4 to 34.3) | p=0.003† | 64.1 (61.0 to 69.3) | NS‡ |
| | Men | 22 | 12.9 (9.1 to 18.5) | | 63.1 (60.7 to 68.6) | |
| 70–79 | All | 19 | 33.9 (24.6 to 46.7)§ | | 74.9 (70.7 to 79.5) | |
| | Women | 11 | 32.4 (19.8 to 52.9) | NS† | 74.3 (70.8 to 79.5) | NS‡ |
| | Men | 8 | 36.1 (21.8 to 59.7) | | 76.8 (70.7 to 79.0) | |
| ≥80 | All | 7 | 83.1 (42.7 to 162)§ | | 83.1 (80.4 to 88.5) | |
| | Women | 4 | 132 (55.3 to 313) | p=0.03† | 83.3 (80.4 to 88.5) | NS‡ |
| | Men | 3 | 45.0 (19.0 to 107) | | 83.1 (82.4 to 86.6) | |

*Geometric mean (95% confidence interval).
**Median (5th to 95th centiles).
§Trend in the plasma concentration of NT-proBNP over age groups analysed by analysis of variance: p<0.0001.
†Two sample *t* test; ‡Mann–Whitney test, women *v* men.
¶Definition of "normal": no congestive heart failure, no ischaemic heart disease, no history of hypertension, no diabetes, no lung disease, no cardiovascular drug treatment, left ventricular ejection fraction ≥60%, blood pressure <140/90, and a normal ECG.

The subjects were also submitted to apical four and two chamber and apical long axis echocardiograms. Left ventricular systolic function was evaluated by 16 segment wall motion score indexing, from which the left ventricular ejection fraction was calculated by multiplying by 30.[13] Heart valves (aortic, mitral, and tricuspid) were evaluated by the colour Doppler technique in apical four and two chamber or long axis echocardiograms. Any sign of stenosis or incompetence led to further assessment with continuous wave Doppler and M mode atrial measurements. Valve defects were characterised on a four grade scale as normal, mild, moderate, or severe. Only subjects with valve defects described as moderate or severe were diagnosed as having valve disease in this study. Two independent and experienced observers evaluated all echocardiograms.

Blood and urine samples were collected for biochemical markers (plasma NT-proBNP, plasma creatinine, plasma glycosylated haemoglobin A1c (HbA1c), and urinary albumin/creatinine ratio). All blood samples were collected with the participant in a sitting position and after at least 15 minutes of rest. Blood samples for analyses of the biochemical markers were immediately centrifuged at 4°C and plasma samples were stored in disposable tubes containing aprotinin (kallikrein inhibitor) in a −80°C freezer.

Plasma concentrations of NT-proBNP were measured by a novel, highly sensitive and specific immunoassay based on a sandwich format using unextracted EDTA plasma. The sensitivity of the assay was < 3.0 pmol/l and the intra-assay and interassay coefficients of variation were 1.3% and 4.8%, respectively.[14]

### "Normal" subjects

Subjects were classified as having a very low probability of heart disease and subgrouped as normal if the following criteria were fulfilled: no congestive heart failure, no ischaemic heart disease, no history of hypertension, no diabetes mellitus, no lung disease, receiving no cardiovascular drugs, and having a left ventricular ejection fraction of ⩾ 60%, a blood pressure of < 140/90 mm Hg, and a normal ECG.

### Statistics

Verification of the normal distribution of continuous data was accomplished using histograms and normal plots. Measures of plasma NT-proBNP, plasma creatinine, urine albumin, and plasma glycosylated haemoglobin showed a log normal distribution and were consequently logarithmically ($\log_{10}$) transformed. Two sample *t* tests were done between mean or geometric mean values of variables in the normal patients and all patients except the normal patients, and between mean or geometric mean values of variables in men and women in case of normal or log normal distribution of data; alternatively Mann–Whitney tests were done. Dichotomous data were analysed by continuity adjusted $\chi^2$ tests. Plasma concentrations of NT-proBNP in the different age groups for each sex were compared using one way analyses of variance. Variables potentially associated with the plasma concentration of NT-proBNP in all subjects, in all subjects except those diagnosed with diabetes mellitus, and in the normal subjects were analysed by multiple linear regression analyses with backward elimination.

All tests were two sided and a significance level of 5% was used. All tests were done using the Statistical Analysis System (SAS Institute, Cary, North Carolina, USA).

## RESULTS

### Basic characteristics and measurements

The criteria for normality, as outlined in the methods section, were met by 130 of the 672 subjects; these were consequently classified as "normal". Clinical characteristics, including fundamental measurements for all subjects and for subjects classified as normal, are given in table 1.

### Variables independently associated with plasma NT-proBNP

In all subjects, female sex, greater age, increasing dyspnoea, diabetes mellitus, valvar heart disease, lower heart rate, left ventricular ejection fraction ⩽ 45%, abnormal ECG, high $\log_{10}$[plasma creatinine], low $\log_{10}$[plasma HbA1c], and high $\log_{10}$[urine albumin] were all independently associated with a

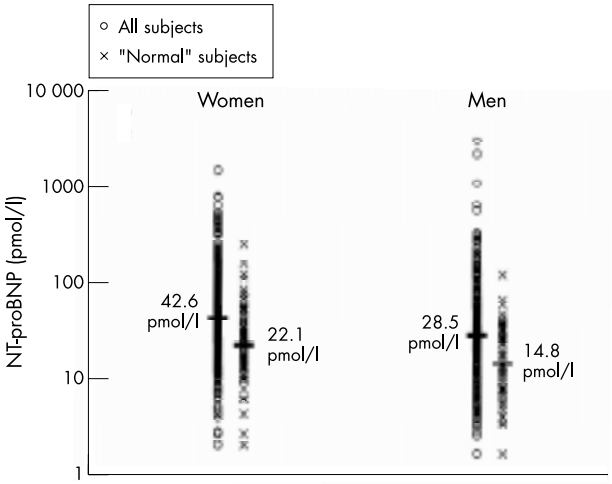


**Figure 1** Plasma concentrations of N-terminal pro brain natriuretic peptide in men and women in the study sample. Horizontal bars indicate geometric mean concentrations. Definition of "normal" subject: no congestive heart failure, no ischaemic heart disease, no history of hypertension, no diabetes, no lung disease, no cardiovascular drugs treatment, left ventricular ejection fraction of ⩾ 60%, blood pressure < 140/90 mm Hg, and a normal ECG. NT-proBNP, N-terminal pro brain natriuretic peptide.

high plasma concentration of $\log_{10}$[NT-proBNP] by multiple linear regression analysis (table 2).

When leaving out subjects diagnosed with diabetes mellitus, the same independent variables were identified: sex (0 = female, 1 = male), regression coefficient (*rc*) (SE) = –0.193 (0.031), p < 0.0001; age (years), *rc* = 0.019 (0.002), p < 0.0001; increasing dyspnoea, *rc* = 0.044 (0.012), p = 0.0002; valvar disease, *rc* = 0.364 (0.117), p = 0.002; heart rate (beats/min), *rc* = –0.005 (0.001), p < 0.0001; left ventricular ejection fraction ⩽ 45%, *rc* = 0.198 (0.052), p = 0.0002; abnormal ECG, *rc* = 0.182 (0.034), p < 0.0001; $\log_{10}$[plasma creatinine (μmol/l)], *rc* = 0.279 (0.081), p = 0.0006; $\log_{10}$[plasma HbA1c (%)], *rc* = –0.600 (0.149), p < 0.0001; and $\log_{10}$[urine albumin (%)], *rc* = 0.078 (0.015), p < 0.0001, intercept (0.163 (0.419), NS).

In the normal subjects, female sex, greater age, and low diastolic blood pressure were independently associated with a high plasma concentration of $\log_{10}$[NT-proBNP] (table 2).

Greater age was by far the most important independent variable in explaining the plasma concentration of $\log_{10}$[NT-proBNP], as in all models it was much more strongly associated with $\log_{10}$[NT-proBNP] than any of the other independent variables.

As sex was also identified as a significant independent variable, plasma concentrations of NT-proBNP in various age and sex groups in all subjects (table 3 and figs 1 and 2) and in the normal subjects (table 4 and figs 1 and 3) were calculated. In all subjects and in the normal subjects, plasma concentrations of NT-proBNP were significantly higher in women than in men, and the mean concentration of plasma NT-proBNP almost doubled per age decade regardless of sex or "normality" status.

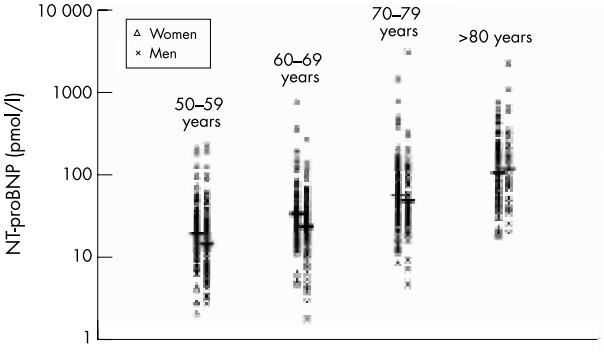


**Figure 2** Plasma concentrations of N-terminal pro brain natriuretic peptide in different age and sex groups in the undivided study sample. Horizontal bars indicate geometric mean concentrations.

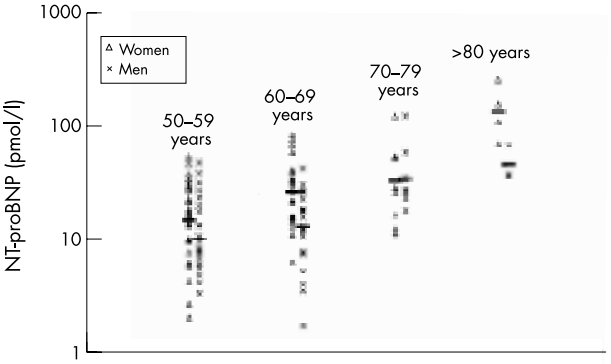


**Figure 3** Plasma concentrations of N-terminal pro brain natriuretic peptide in different age and sex groups in the subjects defined as "normal" in the study sample. Horizontal bars indicate geometric mean concentrations. Definition of normal subject: no congestive heart failure, no ischaemic heart disease, no history of hypertension, no diabetes, no lung disease, no cardiovascular drug treatment, left ventricular ejection fraction of ⩾ 60%, blood pressure < 140/90 mm Hg, and a normal ECG.

## DISCUSSION

This is the first population based study to confirm that the plasma concentration of NT-proBNP increases with age and that it is consistently higher in women than in men. In addition, we have identified several different factors of independent significance for the plasma concentration of the marker.

It has been shown repeatedly that the natriuretic peptides have significant potential as markers for left ventricular systolic impairment and increased left ventricular dimensions, as well as for the clinical syndrome of heart failure. In population based studies, these biochemical markers have proved their legitimacy to such a degree that the European Society of Cardiology has recently included a raised concentration of any one of these peptides in the diagnosis of heart failure.[15]

Focus has concentrated on BNP and its amino terminal portion NT-proBNP, and as these markers are likely to be incorporated into clinical practice within the foreseeable future, an increased understanding of the physiology and pathophysiology of the markers in a general population setting is needed, particularly to establish normal reference intervals.

It is generally accepted that BNP and thus NT-proBNP are mainly released locally from the left ventricle in response to increased wall tension or stretch,[16] accounting for their value as diagnostic markers in heart failure—a condition often characterised by high left ventricular wall stress owing to increased left ventricular dimensions and wall thinning.

The identification of dyspnoea,[17] valvar heart disease,[18–21] a low left ventricular ejection fraction,[7] and an abnormal ECG[22] as variables of independent significance for the value of plasma NT-proBNP in the present study are all readily explicable by this well known association with left ventricular wall stress, and may thus account for the diagnostic value of the marker.

The finding that plasma concentrations of NT-proBNP were higher in women than in men, regardless of age or normality status, has previously been reported in one

population based study,[23] and seems to be an important factor that needs to be taken into account when defining future reference intervals for the marker. The association is, however, not easily explained, though a lower volume of distribution in women than in men could partly account for the difference.

The plasma concentration of NT-proBNP almost doubled per age decade in the present study, regardless of sex or normality status. This association has previously been reported in normal subjects and probably reflects increased myocardial mass,[24] chamber specific alterations in gene expression,[25] and a possible reduction in the renal clearance of natriuretic peptides with aging, not reflected completely by the plasma creatinine concentration.[26]

The finding that a high plasma creatinine was independently associated with a high plasma NT-proBNP in our study came as no surprise, as high natriuretic peptide concentrations have been reported in patients with renal failure[27–30] and in heart failure patients with increased plasma creatinine.[23 31] As well as a reduced clearance of the natriuretic peptides, increased cardiac afterload caused by fluid retention, leading to increased left ventricular wall stress, is a possible explanation for the effect of impaired renal function on the plasma concentration of NT-proBNP.[20 32 33] This may also be part of the reason why a diagnosis of diabetes mellitus was independently related to a high plasma NT-proBNP, as previous studies have shown raised BNP concentrations in diabetic patients with and without microalbuminuria, probably explained by the presence of an early stage of diabetic nephropathy not yet affecting the plasma creatinine level.[34] It was striking that when diabetic subjects were excluded from analysis in our study, a high urine albumin concentration remained independently associated with a high plasma NT-proBNP concentration, probably reflecting the onset of nephropathy.

We have no explanation for the surprising finding of a low glycosylated haemoglobin concentration as a predictor of a high plasma NT-proBNP concentration, nor for the finding that a low heart rate was independently predictive of a high plasma NT-proBNP concentration. The latter association was unaffected by exclusion of subjects on treatment with β blocking agents.

## Conclusions

Our population based results indicate that a single reference interval for normal plasma NT-proBNP will probably not suffice, as adjustments for the independent effects of age and sex appear necessary. In addition, we have identified various other confounders involved in the interpretation of a given plasma NT-proBNP concentration, among which impaired renal function seems to be the most important.

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**Authors' affiliations**

**I Raymond, B A Groenning, P R Hildebrandt, J C Nilsson, F Pedersen,** Department of Cardiology and Endocrinology, Copenhagen University Hospital Frederiksberg, Frederiksberg, Denmark
**M Baumann, J Trawinski,** Integrated Health Care Solutions, F Hoffmann-La Roche Ltd, Basel, Switzerland

## REFERENCES

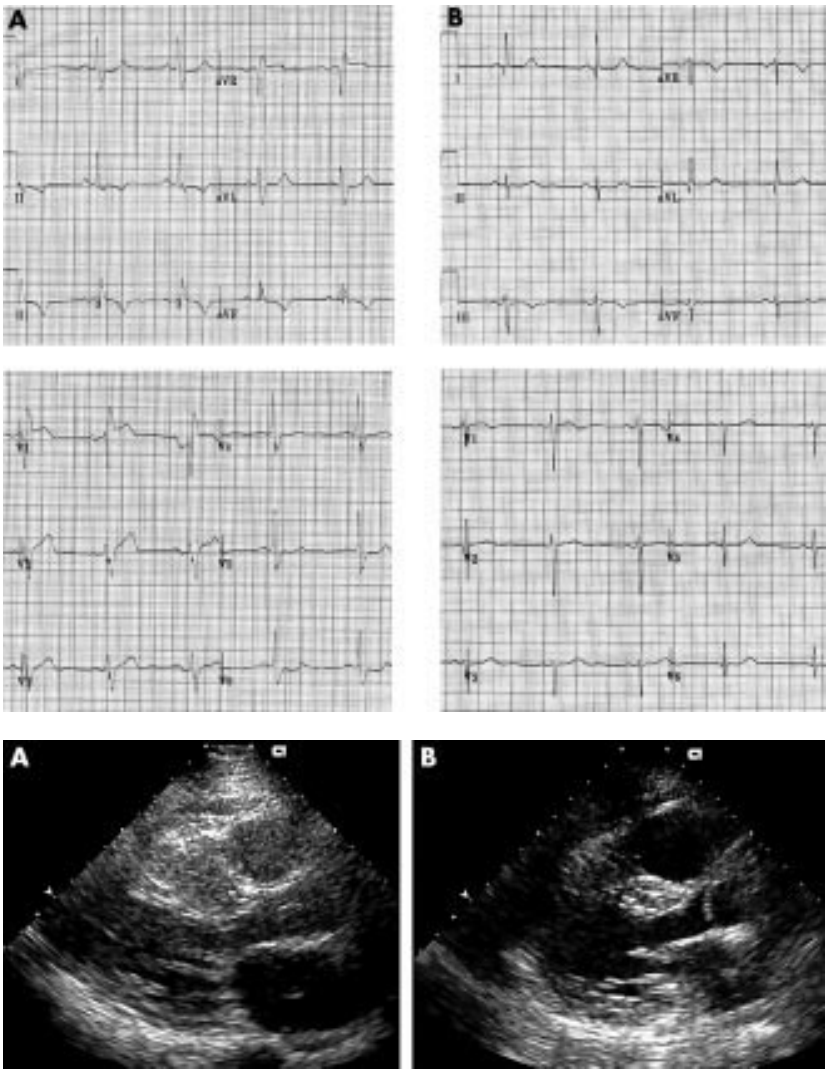
1 **Tsutamoto T**, Wada A, Maeda K, *et al*. Attenuation of compensation of endogenous cardiac natriuretic peptide system in chronic heart failure: prognostic role of plasma brain natriuretic peptide concentration in patients with chronic symptomatic left ventricular dysfunction. *Circulation* 1997;**96**:509–16.
2 **Yamamoto K**, Burnett JCJ, Jougasaki M, *et al*. Superiority of brain natriuretic peptide as a hormonal marker of ventricular systolic and diastolic dysfunction and ventricular hypertrophy. *Hypertension* 1996;**28**:988–94.
3 **Richards AM**, Nicholls MG, Yandle TG, *et al*. Plasma N-terminal pro-brain natriuretic peptide and adrenomedullin: new neurohormonal predictors of left ventricular function and prognosis after myocardial infarction. *Circulation* 1998;**97**:1921–9.
4 **Downie PF**, Talwar S, Squire IB, *et al*. Assessment of the stability of N-terminal pro-brain natriuretic peptide in vitro: implications for assessment of left ventricular dysfunction. *Clin Sci (Colch)* 1999;**97**:255–8.
5 **Richards AM**, Crozier IG, Yandle TG, *et al*. Brain natriuretic factor: regional plasma concentrations and correlations with haemodynamic state in cardiac disease. *Br Heart J* 1993;**69**:414–17.
6 **Magga J**, Vuolteenaho O, Tokola H, *et al*. B-type natriuretic peptide: a myocyte-specific marker for characterizing load-induced alterations in cardiac gene expression. *Ann Med* 1998;**30**(suppl 1):39–45.
7 **McDonagh TA**, Robb SD, Murdoch DR, *et al*. Biochemical detection of left-ventricular systolic dysfunction. *Lancet* 1998;**351**:9–13.
8 **Davidson NC**, Naas AA, Hanson JK, *et al*. Comparison of atrial natriuretic peptide B-type natriuretic peptide, and N-terminal proatrial natriuretic peptide as indicators of left ventricular systolic dysfunction. *Am J Cardiol* 1996;**77**:828–31.
9 **Yamamoto K**, Burnett JC, Bermudez EA, *et al*. Clinical criteria and biochemical markers for the detection of systolic dysfunction. *J Card Fail* 2000;**6**:194–200.
10 **Maisel AS**, Koon J, Krishnaswamy P, *et al*. Utility of B-natriuretic peptide as a rapid, point-of-care test for screening patients undergoing echocardiography to determine left ventricular dysfunction. *Am Heart J* 2001;**141**:367–74.
11 **Cowie MR**, Struthers AD, Wood DA, *et al*. Value of natriuretic peptides in assessment of patients with possible new heart failure in primary care. *Lancet* 1997;**350**:1349–53.
12 **Dao Q**, Krishnaswamy P, Kazanegra R, *et al*. Utility of B-type natriuretic peptide in the diagnosis of congestive heart failure in an urgent-care setting. *J Am Coll Cardiol* 2001;**37**:379–85.
13 **Schiller NB**, Shah PM, Crawford M, *et al*. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography committee on standards, subcommittee on quantitation of two-dimensional echocardiograms. *J Am Soc Echocardiogr* 1989;**2**:358–67.
14 **Karl J**, Borgya A, Gallusser A, *et al*. Development of a novel, N-terminal-proBNP (NT-proBNP) assay with a low detection limit. *Scand J Clin Lab Invest Suppl* 1999;**230**:177–81.
15 **Remme WJ**, Swedberg K. Guidelines for the diagnosis and treatment of chronic heart failure. *Eur Heart J* 2001;**22**:1527–60.
16 **Yasue H**, Yoshimura M, Sumida H, *et al*. Localization and mechanism of secretion of B-type natriuretic peptide in comparison with those of A-type natriuretic peptide in normal subjects and patients with heart failure. *Circulation* 1994;**90**:195–203.
17 **Davis M**, Espiner E, Richards G, *et al*. Plasma brain natriuretic peptide in assessment of acute dyspnoea. *Lancet* 1994;**343**:440–4.
18 **Brookes CI**, Kemp MW, Hooper J, *et al*. Plasma brain natriuretic peptide concentrations in patients with chronic mitral regurgitation. *J Heart Valve Dis* 1997;**6**:608–12.
19 **Prasad N**, Bridges AB, Lang CC, *et al*. Brain natriuretic peptide concentrations in patients with aortic stenosis. *Am Heart J* 1997;**133**:477–9.
20 **Talwar S**, Downie PF, Squire IB, *et al*. Plasma N-terminal pro BNP and cardiotrophin-1 are elevated in aortic stenosis. *Eur J Heart Fail* 2001;**3**:15–19.
21 **Ikeda T**, Matsuda K, Itoh H, *et al*. Plasma levels of brain and atrial natriuretic peptides elevate in proportion to left ventricular end-systolic wall stress in patients with aortic stenosis. *Am Heart J* 1997;**133**:307–14.
22 **Nielsen OW**, Hilden J, Hansen JF. Strong prognostic value of combining N-terminal atrial natriuretic peptide and ECG to predict death in heart patients from general practice. *Heart* 2001; **86**:218–19.
23 **Luchner A**, Hengstenberg C, Löwel H, *et al*. Evaluation of n-terminal pro-brain natriuretic peptide (NT-proBNP) as marker of impaired left ventricular function after myocardial infarction [abstract]. *Eur J Heart Fail* 2000;**2**:47.
24 **Sayama H**, Nakamura Y, Saito N, *et al*. Why is the concentration of plasma brain natriuretic peptide in elderly inpatients greater than normal? *Coron Artery Dis* 1999;**10**:537–40.
25 **Raizada V**, Thakore K, Luo W, *et al*. Cardiac chamber-specific alterations of ANP and BNP expression with advancing age and with systemic hypertension. *Mol Cell Biochem* 2001;**216**:137–40.
26 **Giannessi D**, Andreassi MG, Del Ry S, *et al*. Possibility of age regulation of the natriuretic peptide C-receptor in human platelets. *J Endocrinol Invest* 2001;**24**:8–16.
27 **Akiba T**, Tachibana K, Togashi K, *et al*. Plasma human brain natriuretic peptide in chronic renal failure. *Clin Nephrol* 1995;**44**(suppl 1):S61–4.

28 **Haug C**, Metzele A, Steffgen J, *et al*. Increased brain natriuretic peptide and atrial natriuretic peptide plasma concentrations in dialysis-dependent chronic renal failure and in patients with elevated left ventricular filling pressure. *Clin Investig* 1994;**72**:430–4.
29 **Ishizaka Y**, Yamamoto Y, Fukunaga T, *et al*. Plasma concentration of human brain natriuretic peptide in patients on hemodialysis. *Am J Kidney Dis* 1994;**24**:461–72.
30 **Yandle TG**, Richards AM, Gilbert A, *et al*. Assay of brain natriuretic peptide (BNP) in human plasma: evidence for high molecular weight BNP as a major plasma component in heart failure. *J Clin Endocrinol Metab* 1993;**76**:832–8.
31 **Luchner A**, Burnett JCJ, Jougasaki M, *et al*. Evaluation of brain natriuretic peptide as marker of left ventricular dysfunction and hypertrophy in the population. *J Hypertens* 2000;**18**:1121–8.
32 **Almirez R**, Protter AA. Clearance of human brain natriuretic peptide in rabbits; effect of the kidney, the natriuretic peptide clearance receptor, and peptidase activity. *J Pharmacol Exp Ther* 1999;**289**:976–80.
33 **Nakao K**, Mukoyama M, Hosoda K, *et al*. Biosynthesis, secretion, and receptor selectivity of human brain natriuretic peptide. *Can J Physiol Pharmacol* 1991;**69**:1500–6.
34 **Yano Y**, Katsuki A, Gabazza EC, *et al*. Plasma brain natriuretic peptide levels in normotensive noninsulin-dependent diabetic patients with microalbuminuria. *J Clin Endocrinol Metab* 1999;**84**:2353–6.

# IMAGES IN CARDIOLOGY

## Different presentation of hypertrophic cardiomyopathy in monozygotic twins

A 69 year old woman was referred for cardiac assessment with dyspnoea (New York Heart Association functional class III). Her ECG showed sinus rhythm with right bundle branch block and T wave inversion in inferior leads II, III, and AVF (upper panels, A) An echocardiogram revealed presence of hypertrophic cardiomyopathy (HCM). Interventricular septum was severely thickened at 2.9 cm (lower panel, A). There was severe inducible left ventricular (LV) outflow tract obstruction (gradient > 70 mm Hg, post-intravenous dobutamine infusion). She underwent successful percutaneous ethanol septal ablation. Her monozygotic twin sister (sister B), confirmed by genetic examination, was asymptomatic. Her ECG was different to sister A. There was regression of the R waves in precordial leads and borderline non-specific ST segment changes (upper panels, B). Both sisters had normal blood pressure. An echocardiogram showed no signs of LV outflow tract obstruction and the interventricular septum was only moderately thickened at 1.5 cm (lower panel, B). LV diameters were also different in both sisters (3.8 cm and 4.5 cm, A and B, respectively). However, left atrial size was similar (4.1 cm for both). Both sisters had abnormal LV diastolic indices. Mitral A wave and pulmonary venous flow atrial reversal difference was negative (sister A –60 ms, sister B –35 ms). In both sisters early diastolic tissue Doppler echocardiographic indices were abnormal. Unfortunately, we have not been able to identify the mutation of sarcomeric protein.




These monozygotic twins showed a different phenotypic expression of HCM. Both twins lived far away from each other and had different habits (such as exercise, diet). Sister A had severe LV septal hypertrophy which required treatment while sister B was asymptomatic with only moderate LV hypertrophy. However, both twins had diastolic LV dysfunction. This unique observation on the phenotypic expression in monozygotic twins with HCM supports the proposal that environmental factors may have a significant impact on both the morphological expression and clinical presentation of HCM.

**P Palka**
**A Lange**
**D J Burstow**
ppalka@hotmail.com